Bioremediation of Yamuna River water at Agra with special reference to heavy metals and pesticides using EM technology

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INTRODUCTION

Water is most abundantly available on Earth and required by all kinds of life. An adequate qualitative and quantitative water supply is very important for small as well as big cities and towns. The quality of water is of vital concern for mankind since it is directly linked with human welfare. India is the country which has rich and wide history of social and economic prosperity and of environmental richness. Rivers (Goddesses in Indian Culture) has been facing the curse of pollution for a long time because of unplanned development, urbanization, industrialization, population density and agricultural activities.

Yamuna River is one of the most polluted rivers of India. River Yamuna is the largest tributary of the Ganga River in North India. Pollution level in the Yamuna River has risen due to industrial and domestic effluent discharge into the river through drains. Biological Oxygen Demand (BOD), one of the most important indicator of pollution , load has increased by 2.5 times between 1980 and 2005.The total biochemical oxygen demand content in the Yamuna was 93mg/L, while the permissible level is 3 mg/L. River Yamuna has been reduced to a small stream, draining industrial effluents, sewage, dirt and toxic substances. (**Misra, 2010)**

The pollution of aquatic ecosystem by heavy metals has assumed serious proportion due to their toxicity and accumulative behavior. Metals are introduced into the aquatic system as a result of weathering of rocks from volcanic eruptions and from a variety of human activities involving mining, processing and use of metals or substances containing metal contaminants. **(Jain, 2004)** Various water pollutants and their ill effects on human health are presented in Table-1.

Pollution due to heavy metal ions and pesticides is dangerous as they tend to bioaccumulate and therefore toxic to the plant, human and animal health. Pesticides are the chemicals used in agriculture, in order to protect the crops from the attacks of pests, diseases and rodents. They are toxic and cause environmental contamination as well as generate public health problems. **(Sabale *et al.*, 2012)** The analysis of pesticide residue in water is difficult since these compounds occur at very low concentration. **(Brondi *et al.*, 2005).**

In India alarming levels of pesticides have been reported in air, water, soil as well as in foods and biological material. **(Viswanathan, 1985)** Some of these pesticides have also been reported to be

toxic **(Deflora *et al.*, 1993)**, mutagenic, carcinogenic and tumorogenic **(Rehana *et al.*, 1995)** The DDT content of the Yamuna River which flows through Delhi is one of the highest ever reported, many other problems affect river water quality on a global scale. **(Agrawal *et al.*, 2010)** Organochlorine and organophosphorus pesticides are the most important pollutants among the toxicants in India.

According to 1980 study by the WHO at least 30,000 people die every day in developing countries of the world because of unsanitary water supply.

Table 1: Ill Effects of Water Pollutants

Sr. No.	Pollutants	Effects
1.	Copper (Cu)	Excess Cu in human body is toxic, may cause hypertension, sporadic fever, uremia, coma, causes pathological issue in brain tissue.
2.	Zinc (Zn)	Causes vomiting, renal damage, cramps etc
3.	Barium (Ba)	Excessive Salivation, colic, vomiting, diarrhea, tremors, paralysis of muscles, nervous system, damage to heart and blood vessels
4.	Iron (Fe)	Causes rapid respiration and pulse rates, congestion of blood vessels, hypertension and drowsiness
5.	Cadmium (Cd)	It may cause decrease of red blood cells, impairment of bone marrow, lumber pains, disturbance in calcium metabolism, softening of bones, fractures, skeletal deformation, damage of kidney, hypertension, tumor formation, heart diseases, impaired reproductive function, genetic mutation
6.	Mercury (Hg)	Causes headache, abdominal pain, diarrhea, destruction of haemoglobin, damage of renal tissues, mimamata diseases
7.	Lead (Pb)	Causes brain damage, vomiting, loss of appetite, convulsions, uncoordinated body movements, helplessly amazed state, coma

8.	Radioactive materials/metals/ substances	It causes severe mental retardation and leukaemia in infants. Radioactive metals like heavy metals are nephrotoxic and damage kidneys.
9.	Fluoride	Results in progressive crippling scourge (sponging)/ fluorosis of bones, teeth. May cause metabolic alternations in soft tissues and their functional mechanism.
10.	Chromium (Cr)	It causes cancer, anuria, nephritis, gastrointestinal ulceration, perforation in partition of nose
11.	Manganese (Mn)	Cause growth retardation, fever, sexual impotence, muscles fatigue, eye blindness
12.	Cobalt (Co)	Cause paralysis, diarrhea, low blood pressure, lung irritation, bone effects
13.	Nickel (Ni)	Changes in muscle, brain, lungs, liver, kidney and can also cause cancer, tremor, paralysis and even death
14.	Alkalinity and Acidity	Permissible range of pH value if violated may cause health problem to human and animals and loss of productivity in agriculture
15.	Chlorine (Cl)	Destroys plants and aquatic life and is biocide
16.	Sulphide	Gives bad odour, toxic to many aquatic organisms and animals
17.	Pesticides/Insecticides	Highly poisonous for humans and animals. Also they lower seed germination, plays a role in development of Parkinson's disease, destruction of nerve cells in certain regions of brain resulting in loss of dopamine which is used by nerve cells to communicate with brain. Some of these are physical poisons, some are protoplasmic poisons causing liver damage, some are respiratory poisons and some are nerve poisons
18.	Nitrogen	Can produce a serious condition in fish called "brown blood disease." Causes a condition known as methemoglobinemia or "blue baby" disease.

Different physical and chemical methods were applied to prevent the contamination or clean up the polluted site after the contamination. Among the technologies available to deal with contaminated water or soils, bioremediation based on metabolic activity of microorganisms have certain advantages. **(Exner *et al*., 1994)** Bioremediation is cost effective and efficient method of decontamination that has become increasingly popular now a day to remove environmental population. **(Lessinger., 1996).**

One of those new technologies being proposed is the use of Effective Microorganisms. The concept of effective microorganisms (EM) was developed by Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan **(Higa, 1991; Higa and Wididana, 1991a)**. EM consists of mixed cultures of beneficial an naturally-occurring microorganisms that can be applied as inoculants to increase the microbial diversity of soils and plant.

Studies have shown that EM may have a number of applications, including agriculture, livestock, gardening & landscaping, composting, bioremediation, cleaning septic tanks, algal control & household uses. Research has shown that the inoculation of EM cultures can improve soil quality, soil health, and the growth, yield, and quality of crops. EM contains selected species of microorganisms including predominant populations of lactic acid bacteria (*Lactobacillus* sp) and yeasts (*S.cerivisae*) and smaller numbers of photosynthetic bacteria (*R. palustris*, *R. spheroides*), actinomycetes (*Actinobacteria* sp) and other types of organisms. All of these are mutually compatible with one another and can coexist in liquid culture. The practical application was developed by Professor Teuro Higa. He has devoted much of his scientific career to isolating & selecting different microbes for beneficial effects on soils & plants. He has found microbes that can coexist in mixed cultures & are physiologically compatible with one another. When these cultures are introduced into the natural environment, their individual beneficial effects are greatly magnified in a synergistic fashion **(Crawford, 2002).** Some non culturing bacteria are also found in waste water which has some direct or indirect influence on degradation of pollutants. Bacteria in the Viable but Non culturable state (VBNC) fail to grow on bacteriological media, but these bacteria are alive and have ability of metabolic activity **(Oliver, 2000b)**.

A microbial inoculant containing many kinds of naturally occurring beneficial microbes called ‘Effective Microorganisms’ has been used widely in nature and organic farming **(Diver, 2001).**

However, in biological treatment, the microorganisms degrade the organic pollutants using them as a carbon source to produce metabolic energy to survive.

Hence an attempt has been made to study "Bioremediation of Yamuna River water at Agra with special reference to heavy metals and pesticides using EM technology".

Why selected this problem?

Water is very important for all living organisms including human-beings. Certain water borne diseases like Cholera, Dysentery, Typhoid caused by bacteria which are developed when the water is polluted by different sources like- industries, agriculture, sewage, tannery etc. Water is physically characterized polluted by deposited silt and sullage in the water bodies. The Yamuna river at Agra is highly polluted it is urgent need to control this problem.

Clean water (i.e. the water that is free of toxic chemicals and pathogens) is essential to human health. Clean water is also a critical feed stock in a variety of key industries including electronics, pharmaceuticals and food. The word is facing formidable challenges in meeting rising demands of clean water as the available supplies of fresh water are decreasing due to:-

a. Extended droughts.
b. Population growth.
c. Competing demands of various users.

The water table is going down resulting in decrease in the available water quantity. The hardness of water is increasing day by day and the fluoride as well as chloride contents are found to be very high. Due to shortage of water the society is going through a serious problem and threat to human health and hygiene.

Water purification technology is often complicated and requires sophisticated equipment. It is also expensive to run and maintain. The Effective microbes (EM) technology could prove a simple answer to the problem. EM technology could be used to remove toxic chemicals, bacteria, viruses and other hazardous materials from water much more effectively and at lower cost than other conventional water purification methods.

Organic pollutants from industrial waste water from pulp and paper mills, textiles and leather factories, steel foundries and petrochemical refineries are a major cause of illness in parts of the world where regulations do not necessarily protect the people from such industrial outflows. The

EM approach to water purification could help in preventing diseases and poisoning for potentially millions of people. Use of EM is considered to be economical, energy efficient and environmental friendly with minimal disposal problems. Effective microbes can completely degrade and oxidizes toxic organic compounds; are characterized by low cost and offer the possibility of *in-situ* treatment.

REVIEW OF LITERATURE

PHYSICO-CHEMICAL PARAMETERS

All life on earth depends upon water of the three state in which water occur in the ecosphere as gas, solid and liquid only the last one is an indispensable resource as far as human activity is concerned (Ozha**, 1998**). Sewage is a liquid waste of a community and consists of primarily used water with hardly 0.1 % of solids and is generally considered very good medium for the growth of micro-organisms. This water has to be treated before its disposal and use in agriculture, irrigation etc.

The natural system for waste water treatment have been existing in nature as swamps, bogs, marshes the stimulation of which can be built and used for treatment of waste water. One such method of treating the sewage and making its quality better for its use in various fields is through microbial treatment i.e. inoculation of bacterial strains into the raw sewage and improving the quality of sewage water. The sewage or waste water may harbour many pathogenic organisms and the immediate and greater risk to health might arise due to microbial contaminations of water which invariable result from faecal contamination (Straub *et al.,* **1993**)

Kumar *et al***., (1998)** studied the effect of biodegradation of organic matter in waste water (sewage) as determined by formulated microbial mixture. Various bacterial species such as *Citrobacter, Klebsiella, Yersinia, Serratia,* were isolated from the sewage and initial BOD, COD was calculated. Ultimately it was concluded that sewage may be replaced by formulated microbial mixture for the determination of biodegradable organic matter.

Mishra and Tripathi **(2000)** reported a significant variation in values of DO, BOD, and COD between untreated and treated sewage. The treated sewage showed a sharp reduction in BOD (84.9%) and COD (79.9%) and DO content of treated sewage increased by 62.4%. DO, BOD and COD are important parameters which should be determined to perform the sewage quality analysis. Langwaldt *et al***., (2000)** performed on site biological remediation of contaminated ground water. Various bacterial species such as *Citrobacter,*

Klebsiella, Yersinia, Serratia, were isolated from the sewage and initial BOD, COD was calculated. Dumontet *et al*., **(2001)** have studied the occurrence of viruses, bacteria, yeast and fungi in the sewage and consequential epidemiologic concern have also been reported by these workers.

Joshi and Sharma **(2002)** Performed bioremediation of sewage through bacterial inoculation under *in vitro* and reported a reduction in BOD and COD after 24, 48 and 72 hours. Maximum reduction (79.45% in BOD and 72.25% in COD) was observed after inoculation period of 72 hr. with bacterial cultures.

Ghabjan *et al.,* **(2005)** collected the water samples from different stations of Mulla and Pavana rivers located at Pune, urban area, in Maharashtra, India, to study the monthly variation in physico-chemical parameters of river water.

Water samples were collected from different sampling stations by Toshiniwal *et al*., **(2006)** from Tisgaon Lake in Aurangabad, India for a period of twelve months, during the year June 2004 to May 2005. They studied the monthly variation in water quality parameter i.e. temperature, pH, CO_2, DO, alkalinity, hardness and chloride of lake water.

Kumar and Bhoopathi, **(2007)** collected sago factory effluent from Salem, Tamil Nadu and estimated the physico-chemical parameters by using the consortium of degrading organisms including *Bacillus, Pseudomonas, Cellulomonas* and *Alcaligens* species by inoculating into the effluent.

Meena *et al.,* **(2007)** worked on bioremediation of kitchen wastewater by effective microorganisms. EM involved *Lactobacillus, Rhodopseudomonas, Rhodobacter, Cellulomonas, Nitrobacter and Mucor hiematis*. Reductions in DO, BOD and COD as well as improvement in physicochemical properties were recorded.

Ayyasamy *et al.,* **(2008)** for treatment of sago industry effluents, starch degrading bacteria were isolated. The genera, *Alcaligenes, Bacillus* and *Corynebacterium* were found efficient in starch degradation. The physico-chemical properties (such as BOD, COD, electrical conductivity, dissolved solids, etc,) were found decreased after 72 hrs.

Shrivastava *et al*., **(2010)** reported that the microbes used in EM technology are non- harmful, non-pathogenic, non-genetically engineered or modified and non-chemically synthesized. The bacterial consortium used has *B.subtilis, Cellulomonas* sp*., Lactobacillus* sp*., Rhodobacter spheroides, Rhodopseudomonas palustris.* Microbes originated from their own environment previously exposed to organic substances have greater degradation ability of related waste in biodegradation process.

Raghav *et al*., **(2014)** research work has been carried out to analyze the physico-chemical characteristics of Yamuna river water and to reduce the load BOD, COD, hardness, alkalinity, acidity, dissolved solids, suspended solids, total solids by the process of bioremediation using bacterial consortium. The bacterial consortium treated water sample showed a sharp reduction in BOD i.e. 89% and 84% in COD. The result of the study indicates that Effective bacterial consortium helps in the reduction of water impurities. The observation revealed that the inoculation of bacterial consortium in water may release the nutrients through biodegradation of the organic/inorganic matter of water sources, which promote the plant growth.

HEAVY METALS

Heavy metals are normally regarded as metals with an atomic number 22 to 92 in all groups from period 3 to 7 in the periodic table. Some of the metals such as Cu, Zn, Cd, Pb, Fe, Cr, Co, Ni, Mn, Mo, Se are essential in trace quantities for the general well-being of living organism but an excess of these can be lethal. (Sharma and Rehman, **2009**)

Bubb and Lester **(1991)** had studied the impact of heavy metals on lowland rivers and the implications for man and the environment. Israilli *et al.,* **(1991)** reported that minimum content of Nickel in river water was recorded at upstream of Allahabad and Ettawah respectively, while maximum value of Ni was obtained at downstream of Delhi and Agra.

Mishra *et al.,* **(1994)** assessed heavy metal like Cu, Zn, Pb, Fe, Cr, and Cd for pollution of river Subarnarekha in Bihar. Berg *et al.,* **(1995)** made an attempt to assess the distribution of heavy metals particularly Cr, Cd, Cu, Pb, Mn, Ni, Zn and Se in the lake ecosystem of tropical Lake Kariba, Zimbabwe. *Razo.* Sharma and Agarwal **(1999)** assessed water quality of river Yamuna at Agra.

Mehrotra, **(1999)** investigated pollution of ground water by Mn in Hindon-Yamuna doab (NOIDA area), Dist Gaziabad. Excess loading of hazardous waste has led to scarcity of clean water and disturbances of soil thus limiting crop production (Kamaludeen *et al.*, **2003**)

Sjoblom *et al*., **(2004)** investigated the changes in metal speciation of As, Ca, Cd, Cu, Fe, Pb, and Zn occurring along the river Vormbackenhave.

Hassan *et al.,* **(2010)** conducted study to reveal possible environmental effects on the Euphrates river Iraq and analysed for physical and chemical properties (air and water temperature, pH, electrical conductivity, TDS, TSS and dissolved oxygen) as well as concentration and distribution of some heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in both dissolved and particulate phases in water.

Kaur, **(2012)** assessed the heavy metals concentration of Pb, Fe, Zn, Mn, Cd, Co, Cu, Cr and Ni in the Yamuna river water flowing through the city of Delhi during two seasons viz. summer (May) and winter (January). Similar kind of studies have already carried out by several workers all over the world (Sehgal *et al*., **2012**; Chakrabarty and Sarma, **2011**; Puthiyasekar *et al*., **2010**; Dan'azumi and Bichi, **2010**; Ahmed *et al*., **2010**; Pandey *et al*., **2010**; Borah *et al*., **2009**; Kaushik *et al*., **2009**; Huang *et al.*, **2008**; Gaur *et al*., **2005**; Neal *et al*., **2000**; Zereen *et al*., **1999**; etc.). Pandey *et al.*, **(2013)** reported the concentration of Cr, Cu, Ni, Pb, and Zn which may affect human health of the aquatic ecosystem, in the rivers Ganga and Yamuna on seven sampling stations at Allahabad using Atomic absorption Spectrophotometer. The concentration of these heavy metals in the study area indicated that the river is highly polluted.

Kumar *et al*., **(2013)** study revealed that the heavy metal level were mainly corresponding to the natural background level except for Pb and Ni by increasing human activities in the Yamuna river increase heavy metal load. Trace element accumulation including toxic heavy metals in aquatic ecosystem as a whole is of great importance for the results of pollution level. Based on the results, the distribution of heavy metals in water, sediments and in fish indicated that the concentrations of these metals are derived from water with regular industrial and urban effluents which need to be checked and monitored.

PESTICIDES

Pesticides are the major pollutants of the aquatic environment and their presence is of vital concern because of their potential toxicity towards vertebrates. (Mishra *et al.,* **2006**).

MacRae *et al.,* **(1969)** reported the biodegradation of technical HCH by anaerobic bacteria *Clostridium* sp. Perclich and Lockwood **(1978)** observed the incidence of the pesticides utilizing bacterial genera such as *Bacillus*, M*icrococcus, Pseudomonas* and *vibrio* in the water and sediment samples of irrigational channel. Microbial cleavage is mainly responsible for the degradation of organophosphorus insecticides (Rosenberg and Alexander **1979**). Studies on Actinomycetes, specifically of the genus *Streptomyces* able to oxidize, partially dechlorinate and dealkylate aldrin, DDT and herbicides like metolachlor or atrazine were reported by Ferguson *et al.,* **1977,** Liu *et al.,* **1990, 1991,** Radosevich *et al.,* **1995.** Several bacterial strains such as *Arthrobacter, Pseudomonas, Sphingobium chlorophenolicum,* and *Serratia marcescens* capable of Pentachlorophenol (PCP) degradation have been reported by Edgehill, **1994,** Thakur *et al*., **2002,** Dams *et al.,* **2007**, Singh *et al.,* **2007.**

Sahu *et al.,* **(1992)** investigated the dechlorination of lindane by *Pseudomonas aeruginosa.* Biodegradation of Monocrotophos and organophosphates pesticides by several strains of *Bacillus* and one strain of *Azospirillum lipoferum* were studied by Rangaswamy and Venkateswaralu **(1992)**

Walker *et al.,* **(1993)** reported that the pesticide is mainly degraded by *Pseudomonas* and *Bacillus* and this versatility might be due to the presence of wide range of enzymes. Nawab *et al*., **(2003)** studied the effects of isolated *Pseudomonas* spp. from soil on the DDT, DDD, DDF and HCH under the laboratory conditions. Mohan and Ravichandran **(2010)** analyzed the heterotrophic and pesticide degrading bacteria in sediment samples of Cauvery River bed, where the farmers are using methyl parathions as a potent pesticide in paddy and vegetable fields. Nalin *et al.,* **(1999)** isolated and identify a new bacterial strain *Rhodanobacter lindanclasticus* for degradation of technical grade HCH under aerobic condition. Gupta *et al.,* **(2000)** reported the degradation of Lindane using bacterial culture *Bacillus circulans* and *Bacillus brevis* in 5µg/mL lindane and 80% degradation was noted within 8 days. Manonmani *et al.,* **(2000)** also observed the degradation of the α-HCH isomer by a microbial consortium under a wide range of temperatures (4-40°C) in a liquid culture medium, and 30°C was the optimum for α-HCH degradation. Sreenivasulu and Aparna **(2001)** reported bioremediation of methyl parathion by free and immobilized cells of *Bacillus* sp.

Bhadbhade, **(2001)** extensively studies the microbial degradation of monocrotophos pesticides. The results revealed that *Arthrobacter atrocyaneus, Bacillus megaterium and Pseudomonas mendocina* showed 80 to 90% degradation of monocrotophos at maximum initial concentration of 500 mg/L in synthetic medium within 48 h. Deshpande, **(2002)** reported the degradation of Dimethoate by several bacterial strains. *Brevundimonas* sp. showed 96%, *Bacillus* sp. showed 94% while *Klebsiella oxytoca* showed 71% degradation of dimethoate pesticide within 12 days. Use of endosulfan and methoxychlor has been permitted but HCB was never registered as pesticide. (UNEP, **2003**).

Bioconversion and biological growth kinetics of *Pseudomonas aeruginosa* which degrades HCH was investigated in batch process under aerobic condition by Lodha *et al*., **(2007).** *Pseudomonas aeruginosa* (NCIM 2074) use in bioremediation of chlorpyrifos at concentrations up to 50 mg/l, was reported by Geetha and Fulekar**, 2008.** Bacterial strain *Bacillus thurigiensis* MOS-5 has been used for the biodegradation of malathion by Zeinat *et al.,* **(2008).**

A unique approach for the degradation of organophosphorus pesticide dimethoate in aqueous media with effective microorganisms (EM) was investigated by Megeed and Nakieb **(2008)** and established the EM degradation of dimethoate and suggests their role in the bioremediation of other pesticide contaminated water. In India total 179 pesticides are registered for use, approximately 30 have been banned, while 7 are restricted which includes DDT, Aldrin, Chlordane and heptachlor. Pandey *et al.,* **(2011)** reported the concentration levels and distribution patterns of the organochlorine pesticide residues in the surface sediments of river Yamuna in Delhi. The results revealed contamination of the surface sediments with several persistent organochlorine pesticides. Endrin aldehyde, Endosulfan sulfate and DDT showed the highest percentage in all the three seasons. The total organochlorine pesticides level ranged from 157.71 - 307.66 ng/g in Pre-monsoon to 195.86 - 577.74 ng/g in Monsoon and 306.9 - 844.45 ng/g in the Post-monsoon season. The study demonstrates the pollution of the river with pesticide residues, but also the necessity of a continuous long-term monitoring of the affected environment**.**

Eleni C *et al*., **(2011)** identified two bacterial isolates as *Pseudomonas putida* and *Acinetobacter* to degrade the organophosphate (OP) fenamiphos (FEN).

Kumar *et al.,* **(2014)** reported the distribution of polycyclic aromatic hydrocarbons and organochlorine pesticides in the water and sediments of two urban aquatic bodies. The total PAHs concentration in sediments ranged from 952 to 5353 ng g^{-1} and 649 to 7910 ng g^{-1} for the lakes and river, respectively. The maximum pesticide concentrations in water and sediments of the lakes were 4.12 (aldrin), 2.26 (endrin), 6.17 (pp'-DDT), 5.98 ng mL^{-1}(α-endosulfan) and 167 (aldrin), 124 (endrin), 513 (pp'-DDT), 349 ng g-1 (α-endosulfan), respectively. The aldrin and endrin were absent at all the sites of the Yamuna. The maximum concentration of pp'-DDT and α-endosulfan in river water was 3.37 ng $mL^{-1}$and 3.55 ng mL-1and in sediments 525 ng g-1 and 194 ng g^{-1} respectively.

OBJECTIVES

1. Collection of waste water samples from different sites of Yamuna River at Agra.
2. Isolation and Identification of Culturable and Non-Culturable Bacteria from water samples.
3. Determination of physico-chemical parameters (BOD, COD, Hardness, pH, TS), heavy metals and pesticides from water samples.
4. Optimization of bacterial isolates at different pH, temperature and media.
5. Bacterial treatment (monocultures and consortium) for removal of heavy metal ions and pesticides residue.

METHODOLOGY

1. **Collection of waste water samples from different sites of Yamuna River at Agra**
   Waste Water samples will be collected monthly for one year from different sites of Yamuna River (Kailash Temple, Poiyah Ghat, Hathi Ghat) of Agra region in pre sterilized glass bottles (5 L capacity).

2. **Isolation and Identification of Bacteria from collected water samples**
   Serial Dilution technique would be used for the isolation of bacteria from Yamuna river water samples on nutrient agar medium (Aneja, **2001**). The isolated bacterial strains would be identified on the basis of their morphological, physiological and biochemical characteristics features by Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, **1986**). Isolated bacterial cultures would be cross examined by the BD-BBL Crystal Identification Autoreader for the identification surety. Non-Culturable bacteria would be identified using PCR (Ultee *et al.,* **2004**).

3. **Determination of physico-chemical parameters (BOD, COD, Hardness, pH, TS), heavy metals and pesticides from water samples**
   The initial physico-chemical parameters (color, pH, TDS, BOD, COD, Hardness) of collected water samples would be determined by following the methodology given by Ambasht, **(1990)** and APHA, **(1998)**.
   The heavy metals concentration and pesticides residue in the collected water samples would be determined by Atomic Absorption Spectroscopy (AAS) and Gas Chromatography-Mass Spectroscopy (GC-MS) respectively.

4. **Optimization of bacterial isolates at different pH, temperature and media**
   Optimization of selected bacterial isolates for growth profile at different pH, media and temperature would be carried out by, Saini**, (2008).**

5. **Bacterial treatment (monocultures and consortium) for removal of heavy metal ions and pesticides residue.**
   The efficiency of bacterial monocultures and consortium with respect to reduction in physico-chemical parameters would be carried out following the methodology of APHA **(1998)** and removal of heavy metals and pesticides residue by Atomic Absorption Spectroscopy (AAS) and Gas Chromatography-Mass Spectroscopy (GC-MS) respectively.

SIGNIFICANCE

Water purification technology is often complicated and requires sophisticated equipment. It is also expensive to run and maintain. The Effective microbe technology could prove a simple answer to the problem. Effective microbes could be used to remove toxic chemicals, bacteria, viruses and other hazardous materials from water much more effectively and at lower cost than other conventional water purification methods. Organic pollutants from industrial waste water from pulp and paper mills textiles and leather factories, steel foundries and petrochemical refineries are a major cause of illness in parts of the world where regulations do not necessarily protect the people from such industrial outflows. The Effective microbes approach to water purification could help in preventing diseases and poisoning for potentially millions of people. Biological degradation is environmentally friendly and cost-effective; but it is usually time-consuming.

Use of EM also saves land and capital required for setting up a tertiary plant for cleaning the effluent in some cases. It also helps to reduce pollution caused by crude oil. EM also converts hydrocarbon sulphide and oxide compounds into amino acids, organic oxygen and sugars that fertilize the soil. EM technology is not only environmental friendly, but goes a step forward too actually protect the environment.

EM technology is best and cheapest technology for treatment of waste water. The treated waste water can be used in agriculture and irrigation process. Effective microorganisms convert a degraded ecosystem to one that is productive and contains useful microorganisms.

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